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ORIGINAL ARTICLE

THE EFFECT OF STATIC MAGNETIC FIELD ON BONE MINERAL CONTENT IN AGING RATS

UTICAJ STALNOG MAGNETNOG POLJA NA SADRŽAJ MINERALA KOSTIJU STARIH PACOVA

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Abstract

Introduction: Aging is defined as a consequence of progressive accumulation of metabolic waste, which results in development of various disorders in the structure and function of cells over a number of years. It is followed by loss of bone tissue, where bones become less firm. The cycle of bone remodeling with age becomes longer, and the degree of bone mineralization decreases. Static magnetic fields (SMF) are stable magnetic fields that can be natural or artificial. Their moderate intensity (1mT-1T) affects physiological processes, cells, genetic material, behavior and development. So far, numerous studies have shown different effects of static magnetic fields on cell cultures, experimental animals, and the human population.
Aim: Aim of this study was to determine the effect of static magnetic field (SMF) on bone mineral content (BMC) values in aging rats.
Material and methods: Male Wistar rats, 3 years old, were used in the experiment. A total of 18 animals were divided into two groups: exposed (experimental group) and unexposed (control group). Nine animals from the experimental group were exposed to 30mT intensity static magnetic field for 10 weeks, while nine control rats were not exposed to the static magnetic field. Bone mineral content was measured by DXA (Dual-Energy X-Ray Absorptiometry.
Results: Based on the experiment, it was found that after the exposure to the static magnetic field, in the experimental group, there is a statistically significant increase in the value of BMC in the trunk region and ribs region. In all other regions of interest: head, legs, pelvic bone, spine, total BMC - there were no statistically significant changes in BMC values.
Conclusion: In the conducted experiment, a higher increase in the value of BMC was found in animals exposed to the SMF, compared to animals that were not exposed to the static magnetic field.

Keywords:
aging,
static magnetic field,
bone mineral content

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Sažetak

Uvod: Danas se starenje tumači kao posledica progresivnog nakupljanja razgradnih produkata metabolizma, što može dovesti do nastanka raznih poremećaja u građi i funkciji ćelije tokom niza godina. Starenje je praćeno gubitkom koštanog tkiva, a kosti postaju manje čvrste. Ciklus remodelovanja kostiju starenjem postaje duži, a smanjuje se i stepen mineralizacije kostiju. Stalna magnetna polja (*SMF*) stabilna su magnetna polja koja mogu biti prirodna ili veštačka; *SMF* umerenog dejstva (1mT-1T) utiču na fiziološke procese, ćelije, genetski materijal, ponašanje i razvoj. Do sada su brojna istraživanja pokazala različite efekte stalnih magnetnih polja na ćelijske kulture, eksperimentalne životinje i humanu populaciju.
Cilj: Cilj ovog rada bio je utvrđivanje dejstva stalnog magnetnog polja (*SMF*) na sadržaj minerala kostiju (engl. *Bone mineral content, BMC*) starih pacova.
Materijal i metode: U eksperimentu su korišćeni pacovi soja Vistar, muškog pola, starosti 3 godine. Ukupno 18 pacova podeljeno je u dve grupe: izloženi (eksperimentalna grupa) i neizloženi (kontrolna grupa). Devet pacova iz eksperimentalne grupe izloženo je dejstvu stalnog magnetnog polja (*SMF*) intenziteta 30 mT tokom 10 nedelja, dok 9 pacova iz kontrolne grupe nije bilo izloženo dejstvu stalnog magnetnog polja. Sadržaj minerala kostiju je meren oteodenzitometrijom (*Dual energy X-ray absorptiometry*).
Rezultati: Na osnovu eksperimenta utvrđeno je da, posle izlaganja dejstvu stalnog magnetnog polja, kod eksperimentalne grupe postoji statistički značajan porast vrednosti *BMC* u regionima trupa (*Trunk BMC*) i rebara (*Ribs BMC*). U svim ostalim regionima od interesa: glavi (engl. *Head BMC*), nogama (engl. *Legs BMC*), karličnoj kosti (engl. *Pelvis BMC*), kičmenom stubu (engl. *Spine BMC*), ukupnom *BMC* (engl. *Total BMC*) - nije došlo do statistički značajne promene vrednosti *BMC*.
Zaključak: U sprovedenom eksperimentu utvrđen je veći rast vrednosti *BMC* kod životinja izloženih stalnom magnetnom polju (*SMF*), u odnosu na životinje koje nisu bile izložene stalnom magnetnom polju.

Ključne reči:
starenje,
stalno magnetno polje,
sadržaj minerala kostiju

Introduction

The effects of the magnetic field on human health have been known for a long time. Static magnetic fields (SMF) are stable magnetic fields that can be natural or artificial (directed upwards or downwards). The Earth is surrounded by natural SMF, which varies in intensity between 0.02 and 0.07 mT. Artificial static magnetic field is present in certain industrial processes, MAGLEV achieves 50 mT, medical diagnostic procedures reach doses of 10.5 mT, while small electronic devices reach only a few mT (1). Static magnetic fields are classified as weak (<1mT), moderate (1mT-1T), strong (1-5T) and ultra-strong (>5T). Moderate intensity SMF (1mT-1T) affects physiological processes, cells, genetic material, behavior, and development (2-4).

Aging is a well-documented risk factor for a variety of diseases. Today, aging is interpreted as a consequence of the progressive accumulation of metabolic waste that leads to development of various disorders in the structure and function of the cell over a number of years. More than half of all cancer cases occur in people over the age of 70 (5). Older people are more likely to develop an acute myocardial infarction as well as to die from it (6-7). Also, aging is the most important risk factor for developing neurodegenerative diseases, such as Alzheimer's or Parkinson's disease. Although the science of aging is increasingly focused on biochemical and molecular levels, the influences of environmental factors, different lifestyles, and their interactions with genetics must be taken into account when proposing a particular model of aging (8).

Aging is accompanied by loss of bone tissue, where bones become weaker. The cycle of bone remodeling becomes longer with age, and the degree of bone mineralization decreases. In women, bone density decreases, especially during the early phase of menopause, due to the increased osteoclastic resorption of the mineral matrix caused by the loss of the inhibitory effect of estrogen on osteoclasts. At the age of 70, women lose about 50% of their peripheral cortical bone mass, which leads to deformities, pain, stiffness and fractures. Bone mass also decreases in men, but much later and to a lesser extent than in women. In bone parts that form the joints, osteoporosis also occurs, and such changes cause a higher risk of fractures. As we age, cartilage becomes more rigid, prone to physical trauma and degradation. There is a decrease in the range of motion of the joints (9).

The aim of this study was to determine the effect of static magnetic field (SMF) on bone mineral content (BMC) values in aging rats. Bone mineral content is a measurement of bone mineral content in a specific area in grams (g). It can be measured at a specific location (arm, leg, etc.) or for the whole body. Bone mineral density (BMD) is the amount of bone minerals in bone tissue and is expressed as the quotient of BMC and bone surface area (g / cm^2). Both measurements are significant indicators of osteoporosis and risk of fracture.

Material and methods

Experimental animals

Male Wistar rats, obtained from the vivarium of the Military Medical Academy in Belgrade, were used in the experiment. Total of 18 rats were obtained when they were 8 weeks old and raised until they reached the desired age of 3 years. The individuals were divided into two groups, 9 experimental rats and 9 control rats. Food and water were available to them at will (ad libitum). The individuals were grown under controlled conditions (20 °C, humidity 70%, light-darkness cycle 12h-12h). During the experimental work, the Code of Ethics for Scientific Research of the Faculty of Medicine University of Belgrade was respected, and the examination was approved by the Ethical Committee for Work with Experimental Animals of the Faculty of Medicine University of Belgrade. All experimental protocols were performed according to the procedures described in the Health Guide for Care and Use of Laboratory Animals (Washington, DC, USA).

Magnetic field

The source of SMF was horseshoe magnets made of iron. The intensity of the magnetic field was 30 mT, measured with a digital Teslameter DTM-151 (Group 3 Technology, Auckland, New Zealand) with an accuracy of 0.005 mT. The magnets were placed under cages with rats, which were able to move freely.

Experiment design

A total of 18 animals were divided into two groups: exposed (experimental group) and non-exposed (control group). The exposed group and the unexposed group numbered 9 rats each. The experimental group was exposed to SMF with intensity of 30 mT. Rats were continuously exposed to a constant magnetic field for 10 weeks, and the control group was exposed only to the geomagnetic field. During the experiment, 5 rats died, 3 in the experimental group and 2 in the control group. At the end of the experiment, remaining 6 experimental and 7 control rats were sacrificed under ether anesthesia by cervical dislocation. The experiment analyzed the bone mineral content (BMC-Bone mineral content) in the regions: head (Head BMC), legs (Legs BMC), trunk (Trunk BMC), ribs (Ribs BMC), pelvic bones (Pelvis BMC), spine (Spine BMC) and Total BMC (Total BMC). It was measured at the beginning and at end of the experiment. The experiment was performed at the Clinic for Rheumatology of the Military Medical Academy in Belgrade.

BMC measurement

Bone mineral content was measured by DXA method (Dual energy X-ray absorptiometry). A device called InAlyzer was used, manufactured by MEDIKORS (Jungwon, Seongnam, Korea).

Statistical analysis

Program used for testing was IBM SPSS Statistics V21.0. The difference between the groups was assessed by the Wilcoxon signed rank test. Statistical significance was set at $p < 0.05$.

Results

Based on the experiment, it was determined that, after exposure to SMF, in the experimental group there was a statistically significant increase in value of BMC in regions of the trunk and ribs. In all other regions of interest: head, legs, pelvic bone, spine, total BMC - there were no statistically significant changes in BMC values. In the control group, there was a statistically significant increase in value of BMC in the regions of the ribs and the spine. In other regions of interest: head, legs, trunk, pelvis, total BMC - there were no statistically significant changes in BMC values.

In the Head region, a slight increase in mean BMC values was observed in both the experimental and control group. This growth was not statistically significant in the experimental group ($p = 0.168$), neither in the control group ($p = 0.310$). In the Legs region, there is a very slight increase in mean BMC value in the experimental group that was not statistically significant ($p = 0.916$), as well as a change in mean BMC value in control group that was statistically insignificant ($p = 0.673$). In the Trunk region, a more significant increase in mean value of BMC was observed in the experimental group which was statistically significant ($p = 0.046$), compared to the control group where the growth of mean BMC value was mild and not statistically significant ($p = 0.202$). In the Ribs region, statistically significant increase in mean value of BMC was observed in the experimental group ($p = 0.046$), while in the control group the growth was milder and also statistically significant ($p = 0.027$). In the Pelvic region, a similar slight increase in the mean BMC value was observed in the experimental so as in the control group. This slight growth was statistically insignificant in the experimental group ($p = 0.581$) so as the control group ($p = 0.395$). In the Spine region, a moderate increase in mean BMC value was observed in the experimental group that was statistically insignificant ($p = 0.058$), while in the control group, an increase in mean BMC value was statistically significant ($p = 0.04$). In case of Total BMC, we observe a higher increase in the mean value of BMC in the experimental group compared to the control group. Growth was statistically insignificant in the experimental group ($p = 0.116$), so as the control group ($p = 0.237$).

In all regions of interest, except the pelvic bone, there is a higher percentage increase in mean value of BMC in the experimental group compared to the control group. In the Head region, the experimental group showed

Table 1. BMC values in control and experimental group, before and after exposure to 30mT intensity static magnetic field

	Experimental group			Control group		
	N	Mean±SD	p	N	Mean±SD	p
Head(B)	6	2.5±0.3	0.168	7	2.4±0.4	0.310
Head(E)	6	2.7±0.4		7	2.5±0.2	
Legs(B)	6	2.4±0.3	0.916	7	2.9±0.3	0.673
Legs(E)	6	2.5±0.5		7	2.9±0.5	
Trunk(B)	6	5.1±1.4	**0.046***	7	5±1	0.202
Trunk(E)	6	6.4±1.1		7	5.2±1	
Ribs(B)	6	1.4±0.4	**0.046***	7	1.4±0.4	**0.027***
Ribs(E)	6	2.1±0.4		7	1.6±0.5	
Pelvis(B)	6	1.8±0.5	0.581	7	1.6±0.2	0.395
Pelvis(E)	6	1.9±0.3		7	1.7±0.3	
Spine(B)	6	2±0.5	0.058	7	2±0.5	**0.040***
Spine(E)	6	2.4±0.5		7	2.2±0.5	
Total BMC(B)	6	10.1±1.9	0.116	7	10.4±1.7	0.237
Total BMC(E)	6	11.7±1.8		7	10.9±1.5	

Table 1. Showing the number of experimental animals (N), mean BMC (Mean), standard deviations (SD) and p-values. The letter "B" indicates the first measurement (Beginning) performed at the beginning of the experiment, while the letter "E" indicates the second measurement (End) performed at the end of the experiment.
* Statistically significant difference ($p < 0.05$)

an increase of 8% in mean BMC value, while the control group recorded an increase of 4.2%. In the Legs region, the experimental group showed an increase in mean value of BMC by 4.2%, while in the control group there was no increase in BMC (0%). In the Trunk region, the experimental group recorded an increase in mean BMC value by 25.5%, while the control group recorded an increase of 4%. In the Ribs region the experimental group showed an increase in mean value of BMC by 50%, while there was an increase of 14.2% in control group. In the Pelvic region the experimental group recorded an increase in mean value of BMC by 5.6%, while there was an increase of 6.3% in control group. In the Spine region, the experimental group recorded an increase in mean BMC value by 20%, while the control group recorded an increase of 10%. Observing the change in Total BMC in the experimental and control groups, higher increase in mean value of BMC was observed in the experimental group - 15.8%, compared to the control group where at the end of the measurement the mean BMC value increased by 4.8%.

Discussion

Our results show that after being exposed to a static magnetic field (SMF), there is a statistically significant increase in BMC values in the Ribs and Trunk regions, in the experimental group. In the regions: Head, Legs, Pelvis, Spine and Total BMC, there were statistically insignificant changes in BMC value after exposure to static magnetic field. Although no statistically significant changes in BMC values were observed in all regions of interest, a trend of increasing BMC values after exposure to a static magnetic field was noted in all regions (**table 1**, **table 2**). Comparing the Total BMC values in experimental and control group, a more significant increase in the BMC value in the experimental group was noticed, compared to the control group. Although the growth of BMC was not statistically significant in the experimental or in the control group, the percentage growth was higher in the experimental group (15.8%) compared to the control group (4.8%) (**table 1**).

Previous research also states that static magnetic field has a positive effect on bone mineral content. In the research work of Taniguchi et al. (10) a statistically significant increase in BMC was observed in animals exposed to SMF compared to non-exposed animals. In both groups, osteopenia was previously induced by ovarian removal. Ovariectomy is performed to stop the protective effect of estrogen on bone tissue. Estrogen acts on osteoclasts by inhibiting bone resorption. By removing the ovaries, a postmenopausal condition was achieved. The aim of this study

Table 2. Growth of mean BMC value during the experiment

	Experimental group	Control group
Head BMC	8.0%	4.2%
Legs BMC	4.2%	0.0%
Trunk BMC	25.5%	4.0%
Ribs BMC	50.0%	14.2%
Pelvis BMC	5.6%	6.3%
Spine BMC	20.0%	10.0%
Total BMC	15.8%	4.8%

was to investigate the potential therapeutic application of SMF in the treatment of osteoporosis. The mechanism of action of SMF can be the following: magnetic force increases the release of acetylcholine (ACh) from cholinergic vasodilator nerve endings by inhibiting the effects of cholinesterase, which causes vasodilation. Achieved vasodilation which leads to better bone nutrition, results in an increase in BMC (10). The effects of 1mT SMF administration over 10 minutes on the cutaneous microcirculatory system led to increased vasodilation and vasomotor in blood vessels with increased vascular tone, due to the action of norepinephrine (NA) (11). In blood vessels with reduced vascular tone due to the action of acetylcholine (ACh), there was vasoconstriction and a decrease in vasomotor. In summary, these phenomena suggest that SMF may modulate vascular tone by biphasic modification of vasomotor in skin tissue (11).

A study conducted by Xu S et al. (12) reported that the effect of SMF significantly increased BMC values in osteoporotic lumbar vertebrae in ovariectomized rats. It is stated that the growth of BMC under the influence of SMF most likely occurs as a consequence of increased blood circulation in the bones and bone marrow. It is possible that the increased blood flow through the bone under the influence of SMF affects the better supply of bone tissue with growth factors such as BMP (Bone morphogenetic protein). It induces bone and cartilage formation, thus increasing BMC values. Other research has also supported the theory that SMF increases BMC values by promoting the formation of new and regeneration of old blood vessels, and thus increases collateral circulation (13, 14). Research work of Xu S et al. (12) states that reduced BMC values, caused by artery ligation and tissue ischemia, can be restored by implanting a magnetic rod into the bone itself, which emits a constant magnetic field (13-15). Collateral circulation was measured by injecting microspheres into the abdominal aorta 3 weeks after ligation, where it was observed that bone with an implanted magnetic rod retained a much larger number of microspheres than bone without a magnetic rod. This indicates the previously mentioned effect of static magnetic field on the increase of microcirculation, as well as on the increase of collateral circulation (14).

The differences between our study and other studies stem from the different design of the experiment. We used male Wistar rats. In other experiments, female Wistar rats were used (10, 12). In our experiment, the experimental animals were 3 years old and the intensity of the static magnetic field was 30mT. In the said experiment, conducted by Taniguchi et al. (10), age of the animals was 8 weeks, and the intensity of magnetic field was 200mT. In the experiment conducted by Xu S et al. (12) age of the animals was 10 weeks, and the intensity of the magnetic field was 180 mT. Limitation of our study is the smaller number of experimental animals, which makes it difficult to record a statistically significant change between measurements. Our study is the first study ever performed that used naturally aged animals, rather than induced osteopenia, to examine the effects of static magnetic field on bone mineral content (BMC).

Effects of SMF do not depend on electricity, so during therapeutic use there is no danger of harmful effects of electricity, as well as the danger of burns. For these reasons, SMF can potentially be used for therapeutic purposes as a form of long-term topical therapy (12).

Conclusion

In the conducted experiment, a higher increase in value of BMC was found in animals exposed to static magnetic field, compared to animals that were not exposed to static magnetic field. In two regions of interest: trunk and ribs there was a statistically significant increase in BMC, while in other regions of interest: head, legs, pelvic bone, spinal column and total BMC there was statistically insignificant increase in BMC, but there is a trend of growth in mean value of BMC in all regions of interest that were exposed to SMF. The increase in BMC, as a consequence of the effect of SMF on bone, is probably due to an increase in local microcirculation. This effect of SMF can potentially be used for therapeutic purposes.

References

1. Feychting M. Health effects of static magnetic fields--a review of the epidemiological evidence. Prog Biophys Mol Biol. 2005;87(2-3):241-6.
2. Miyakoshi, Junji. “Effects of static magnetic fields at the cellular level.” Progress in biophysics and molecular biology 87.2-3 (2005): 213-23.
3. Saunders R. Static magnetic fields: animal studies. Prog Biophys Mol Biol. 2005;87(2-3):225-39.
4. Ghodbane S, Lahbib A, Sakly M, Abdelmelek H. Bioeffects of static magnetic fields: oxidative stress, genotoxic effects, and cancer studies. Biomed Res Int. 2013; 2013, 602987.
5. Aunan JR, Cho WC, Søreide K. The Biology of Aging and Cancer: A Brief Overview of Shared and Divergent Molecular Hallmarks. Aging Dis. 2017;8(5):628-42.
6. Niccoli T, Partridge L. Ageing as a risk factor for disease. Curr Biol. 2012;22(17):R741-R752.
7. Shih H, Lee B, Lee RJ, Boyle AJ. The aging heart and post-infarction left ventricular remodeling. J Am Coll Cardiol. 2011;57(1):9-17.
8. Rakic A, Milovanovich I, Trbovich A, Stefanović S, Nikolić D, Janković S et al. Trace elements in different tissues in aging rats, Journal of Trace Elements in Medicine and Biology, 2020; 62, 126604.
9. Beleslin, Čemerikić, Cvetković, Đorđević, Đorđević-Denić, Marković et al. Specijalna patološka fiziologija: Promena skeletnog i mišićnog sistema u procesu starenja. II izdanje. Beograd: Data Status; 2008.
10. Taniguchi N, Kanai S. Efficacy of static magnetic field for locomotor activity of experimental osteopenia. Evid Based Complement Alternat Med. 2007;4(1):99-105.
11. Okano H, Ohkubo C. Modulatory effects of static magnetic fields on blood pressure in rabbits. Bioelectromagnetics. 2001 Sep; 22(6):408-18.
12. Xu S, Okano H, Tomita N, Ikada Y. Recovery Effects of a 180 mT Static Magnetic Field on Bone Mineral Density of Osteoporotic Lumbar Vertebrae in Ovariectomized Rats. Evid Based Complement Alternat Med. 2011; 2011:620984
13. Xu S, Tomita N, Ohata R, Yan Q, Ikada Y. Static magnetic field effects on bone formation of rats with an ischemic bone model. Biomed Mater Eng. 2001;11(3):257-263.

14. Xu S, Tomita N, Ikeuchi K, Ikada Y. Recovery of small-sized blood vessels in ischemic bone under static magnetic field. Evidence-Based Complementary and Alternative Medicine. 2007; 4(1): 59-63.
15. Yan Q C, Tomita N, Ikada Y. Effects of static magnetic field on bone formation of rat femurs. Medical Engineering and Physics. 1998; 20(6): 397-402.